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# Interactions of Heavy Metals with the Pulmonary Metabolism of (3H) Benzo(a)pyrene

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16. ABSTRACT

To test the hypothesis that the inhalation of heavy metal aerosols from polluted air or cigarette smoke could alter the metabolism of polycyclic aromatic hydrocarbons, isolated, ventilated, and perfused rat lungs were used to study the effects of $NiCl_2$, $CdCl_2$, and $CoCl_2$ on the pulmonary metabolism of [$^3$H]benzo[*a*]pyrene ([$^3$H]BAP). Five minutes prior to removing the lungs from a rat, 100 μl of isotonic sucrose containing 1 μmole of $NiCl_2$, $CdCl_2$, or $CoCl_2$ was instilled intratracheally. The lungs were placed in the perfusion system and washed free of blood. After 10 min, 5 nmole of [$^3$H]BAP dissolved in 100 μl of dimethylsulfoxide was introduced in the arterial circulation. After 45 min, the lungs were homogenized and the homogenates and perfusates were extracted with ethyl acetate:acetone (2:1, v/v) for analysis by HPLC. Binding of [$^3$H]BAP metabolites to lung tissue was also determined. The metabolism of [$^3$H]BAP was found to be inducible by pretreatment of rats with β-naphthoflavone (80 mg/kg) and to be inhibited by the inclusion of metyrapone (1.0 mM) or indomethacin (0.1 mM) in the perfusate. Instillation of $NiCl_2$ resulted in a slight inhibition of metabolism. In the presence of $CdCl_2$, 20% more of the parent compound was metabolized compared to sucrose-treated controls, but the fraction of organic solvent extractable metabolites increased to 213% of controls. $CoCl_2$ was without effect on [$^3$H]BAP metabolism. In all cases, the extent of covalent binding to lung macromolecules was proportional to the extent of metabolism of the [$^3$H]BAP. Since heavy metals and polycyclic aromatic hydrocarbons coexist in the same atmospheric aerosols, heavy metal exposure from polluted urban or occupational air or cigarette smoking may have profound effects upon the bioactivity of a common organic carcinogen.

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# Interactions of Heavy Metals with the Pulmonary Metabolism of [$^3$H]Benzo[a]pyrene[1]

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To test the hypothesis that the inhalation of heavy metal aerosols from polluted air or cigarette smoke could alter the metabolism of polycyclic aromatic hydrocarbons, isolated, ventilated, and perfused rat lungs were used to study the effects of $NiCl_2$, $CdCl_2$, and $CoCl_2$ on the pulmonary metabolism of [$^3$H]benzo[*a*]pyrene ([$^3$H]BAP). Five minutes prior to removing the lungs from a rat, 100 μl of isotonic sucrose containing 1 μmole of $NiCl_2$, $CdCl_2$, or $CoCl_2$ was instilled intratracheally. The lungs were placed in the perfusion system and washed free of blood. After 10 min, 5 nmole of [$^3$H]BAP dissolved in 100 μl of dimethylsulfoxide was introduced in the arterial circulation. After 45 min, the lungs were homogenized and the homogenates and perfusates were extracted with ethyl acetate:acetone (2:1, v/v) for analysis by HPLC. Binding of [$^3$H]BAP metabolites to lung tissue was also determined. The metabolism of [$^3$H]BAP was found to be inducible by pretreatment of rats with β-naphthoflavone (80 mg/kg) and to be inhibited by the inclusion of metyrapone (1.0 mM) or indomethacin (0.1 mM) in the perfusate. Instillation of $NiCl_2$ resulted in a slight inhibition of metabolism. In the presence of $CdCl_2$, 20% more of the parent compound was metabolized compared to sucrose-treated controls, but the fraction of organic solvent extractable metabolites increased to 213% of controls. $CoCl_2$ was without effect on [$^3$H]BAP metabolism. In all cases, the extent of covalent binding to lung macromolecules was proportional to the extent of metabolism of the [$^3$H]BAP. Since heavy metals and polycyclic aromatic hydrocarbons coexist in the same atmospheric aerosols, heavy metal exposure from polluted urban or occupational air or cigarette smoking may have profound effects upon the bioactivity of a common organic carcinogen.

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous components of atmospheric particles and constituents of tobacco smoke which are carcinogenic in laboratory animals (Heidelberger, 1975; Kapitulnik *et al.*, 1977) and are mutagenic in bacterial test systems (Nagao and Sugimura, 1979) and in mammalian cell cultures (Maher and McCormick, 1979). Epidemiological evidence suggests that exposure to PAH is a major determinant in human pulmonary carcinogenesis, especially in smokers.

Heavy metals are also constituents of atmospheric particles produced from fossil fuel combustion. The concentrations of heavy metals in airborne particles vary enormously in both space and time. However, the average annual urban

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concentrations of Ni, Cd, and Co are about 0.06, 0.01 and 0.006 $\mu g/m^3$, respectively (Lee *et al.*, 1972). Peak concentrations of these elements in urban atmospheres may reach 10 times these averages and industrial exposure may be 1000 times greater than ambient levels. The quantity of heavy metals to which a person is exposed chronically may be influenced greatly by cigarette smoking. A person smoking one pack of filter cigarettes per day could inhale an additional 2 $\mu g$ of cadmium, 0.4 to 1.6 $\mu g$ of nickel, and 1 to 7 $\mu g$ of zinc (Menden *et al.*, 1972). Even greater amounts of these metals would be released into the atmosphere in side-stream smoke, increasing the dose received by the smoker as well as exposing n[illegible] smokers in the vicinity.

The toxicity of heavy metals and PAH may be potentiated because of the physical characteristics of the particle with which they are associated. The heavy metal content of fly ash from coal fired power plants is inversely proportional to particle size (Natusch and Wallace, 1974). As a result, nickel, zinc, chromium, and cadmium may be four to eight times more concentrated in the particles of respirable size [equivalent aerodynamic diameter (EAD) 1–2 $\mu m$] as compared to nonrespirable particles (EAD > 10 $\mu m$). Thus, the concentrations of heavy metals in particles which are respirable are much greater than those found in whole fly ash samples. Inhalation represents an efficient method of exposure also. Moreover, the distribution of metals among particles in the same size fraction is heterogenous with a relatively small number of particles containing the bulk of the trace metal (Hayes *et al.*, 1978). The local tissue dose on inhalation, therefore, could be high for a relatively small number of cells.

PAH exhibit a pattern of distribution similar to that of heavy metals. The majority of the atmospheric burden is associated with the particles less than 2 $\mu m$ in diameter (Pierce and Katz, 1975). The concentration of total PAH in the atmosphere ranges from 0.1 to 100 $ng/m^3$. However, wide regional and seasonal variation is found in both the concentration and distribution of atmospheric PAH (Baum, 1978).

The lung, and other tissues, can metabolize benzo[*a*]pyrene (BAP) and other PAH to a variety of more polar compounds for detoxification and excretion or to form biologically active intermediates. The ability of a PAH to act as a carcinogen or mutagen is related to the formation of these reactive intermediates (epoxides) and to the ability of the tissue to detoxify these compounds by oxidation or conjugation, factors which are highly tissue specific (Gelboin, 1969; Kinoshita and Gelboin, 1972).

Heavy metals potently affect MFO reactions in many *in vitro* test systems. For example, in the microsomal fraction from hamster fetal cell cultures, $Ni^{2+}$, $Mn^{2+}$, $Co^{2+}$, and $Mg^{2+}$ stimulate, while $Fe^{2+}$, $Zn^{2+}$, $Sn^{2+}$, and $Cd^{2+}$ inhibit, BAP hydroxylase (Nebert *et al.*, 1973). These effects are independent of the source of supply of NADPH, suggesting that these ions act directly upon the microsomal oxidase.

This paper describes the interactions of heavy metals with the pulmonary metabolism of [$^3$H]BAP. The isolated, ventilated, and perfused lung (IVPL) is employed to maintain the complex structure of the lung. Since heavy metals may interact at several points in the multicomponent system responsible for biotrans-

formation of xenobiotics, metabolism in whole organs may be more illustrative than microsomal preparations of the effects which occur *in vivo*. BAP was chosen as a model substrate since its metabolism is well defined and it is one of the most abundant of the carcinogenic PAH species in the environment.

## MATERIALS AND METHODS

*Chemicals*. 1,3,6-[$^3$H]BAP (66.3 Ci/mmole) was purchased from New England Nuclear Corporation, Boston, Massachusetts. Unlabeled BAP metabolite standards, (±)-*trans*-9,10-dihydrobenzo[*a*]pyrene-9,10-diol (9,10-BAP-diol), (±)-*trans*-4,5-dihydrobenzo[*a*]pyrene-4,5-diol (4,5-BAP-diol), (±)-*trans*-7,8-dihydrobenzo[*a*]pyrene-7,8-diol (7,8-BAP-diol), benzo[*a*]pyrene-6,12-dione (6,12-BAP-dione), 9-hydroxybenzo[*a*]pyrene (9-OH-BAP), and 3-hydroxybenzo[*a*]pyrene (3-OH-BAP), were obtained through Dr. David Longfellow, Director of the Information and Resources Segment, National Cancer Institutes, Bethesda, Maryland. Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) and β-naphthoflavone (BNF) were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Indomethacin, D-saccharic acid-1,4-lactone, aryl sulfatase (Type VIII, EC 3.1.6.1), and β-glucuronidase (Type VII) were purchased from Sigma Chemical Company, St. Louis, Missouri. All other chemicals used were of reagent grade and were obtained from standard sources.

*Animals*. Adult male Sprague–Dawley rats (176–199 g) were purchased from Harlan–Sprague–Dawley, Madison, Wisconsin. Rats were raised on hardwood bedding and allowed water and Purina Lab Chow *ad libitum*. No rats were used less than 3 days after arrival and all were used within 7 days.

*Preparation of isolated, ventilated, and perfused rat lungs*. Lungs were removed from anesthetized rats (sodium pentobarbital, 35 mg/kg) as described previously (Williams *et al.*, 1980). The lungs were placed in an artificial thorax and perfused with Krebs–Henseleit bicarbonate buffer containing 4% BSA (Fraction V powder, Sigma Chemical Co.) and equilibrated with 95% $O_2$:5% $CO_2$ in a glass gas exchange column to maintain a pH between 7.35 and 7.45. Lungs were ventilated by positive pressure with humidified room air (80 breaths/min, tidal volume 1.5 $cm^3$, and end expiratory pressure 3 cm $H_2O$). The thorax, perfusate reservoir, and arterial perfusion lines were water jacketed to maintain a constant temperature of 37°C. The lungs were washed initially with 60 ml of the perfusate, which was discarded. The perfusion was then switched to a recirculating system of constant volume (50 ml), with a flow rate of 10 ml/min.

*Metabolism of [$^3$H]BAP in the isolated, ventilated, and perfused lung*. After 5 min of perfusion, 5 nmole of [$^3$H]BAP dissolved in 100 μl of dimethylsulfoxide was infused into the arterial circulation over a period of 1 min. After 45 min, the lungs were removed from the system trimmed of nonpulmonary tissue, blotted dry, weighed (1.1–1.3 g), and immediately frozen at −70°C. All experiments and manipulative procedures were performed under subdued yellow-gold fluorescent lamps to prevent photodegradation of [$^3$H]BAP.

*Extraction of [$^3$H]benzo[a]pyrene metabolites from tissue and perfusate*. The lungs were homogenized in 10 vol of ice cold 1.15% KCl with a Polytron (Brinkman Instruments, Westbury, N.Y.) and brought to a final volume of 25 ml.

The homogenates and perfusates were extracted two times with 2 vol of ethyl acetate:acetone (2:1, v/v). Aliquots (200 μl) of the homogenate were taken prior to and after extraction, digested in Scintigest (Fisher Scientific Co., Pittsburgh, Pa.), and counted by liquid scintillation spectroscopy. Aliquots (100 μl) of the perfusate prior to and after extraction also were counted. The extraction efficiency of [$^3$H]BAP by this method was 99%. Counting efficiency was determined by the external standard channels ratio method. The ethyl acetate:acetone extract was dried over $NaSO_4$, evaporated to a small volume under a vacuum and brought to dryness under a gentle stream of $N_2$. The residue was dissolved in 1.25 ml of acetonitrile for HPLC analysis. In all cases, 85–90% of the original [$^3$H]radioactivity was accountable as extractable tissue and perfusate metabolites, $^3H_2O$ and $^3H$ bound to tissue.

*Analysis of conjugated metabolites of benzo[a]pyrene.* Aliquots (5 ml) of the previously extracted perfusates and lung homogenates were incubated at 37°C with 0.2 mg aryl sulfatase (low in β-glucuronidase activity). Residual β-glucuronidase activity was inhibited by the addition of 0.1 mM D-saccharic acid-1,4-lactone in 5.0 ml of 0.2 M acetate buffer (pH 5.0). Other aliquots were treated with 0.2 mg β-glucuronidase in 5 ml of 0.1 M phosphate buffer (pH 7.0). Control extracts were incubated with buffer solutions alone. After 2 hr of incubation each sample was extracted and evaporated as before and the residues were dissolved in 0.25 ml of acetonitrile for HPLC analysis.

*High pressure liquid chromatographic analysis of [$^3$H]benzo[a]pyrene metabolites.* Chromatographic analysis was performed at room temperature using a Beckman/Altex Model 332 gradient chromatograph equipped with a 25-cm ultrasphere ODS column. Metabolites were eluted with an initial solvent composition of 50% acetonitrile in water at a flow rate of 0.8 ml/min. After 5 min, the acetonitrile concentrations was increased linearly to 70% over a period of 10 min. After 10 additional min. the acetonitrile concentration was increased again to 100% over 15 min and remained at 100% for the duration of the elution.

Radiochromatograms of [$^3$H]BAP metabolites extracted from lungs and perfusates were quantitated by collecting fractions (0.4 ml) of the eluent directly from the column into scintillation vials. Recovery from the column was 100%. A typical chromatogram is shown in Fig. 1. The compounds which were collected in the first 7.5 min of the elution were designated as the polar fraction, since these metabolites elute prior to the 9,10-BAP-diol standard. The compounds which eluted between 7.5 and 11.5 min were designated as diol group 1. The major fraction of radioactivity cochromatographed with the 9,10-BAP-diol reference standard. The group of metabolites which eluted between 11.5 and 22 min was designated as diol group 2 because these cochromatograph with the 7,8-BAP-diol and 4,5-BAP-diol standards. Metabolites which eluted between 23 and 29 min were designated as quinones, since 6,12-BAP-dione exhibits a retention time of 28 min. Phenols were identified as the metabolite fraction appearing between 31 and 43 min, since this broad peak cochromatographs with 9-hydroxy-BAP and 3-hydroxy-BAP. BAP eluted between 45 and 47 min.

Conjugate formation was quantitated by subtracting the radioactivity in chromatographic peaks of buffer-incubated extracts from those of enzyme-incubated samples. The residual BAP peak was used to normalize the two chromatograms.

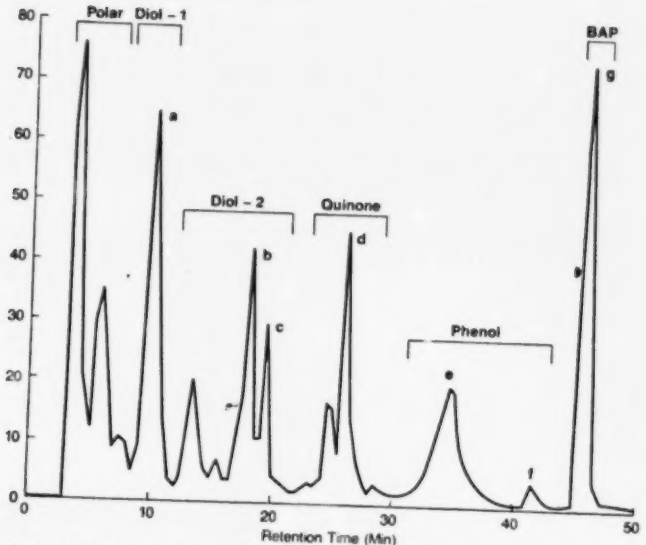


FIG. 1. Typical radiochromatogram of [$^3$H]BAP metabolites extracted from the perfusate of a control IVPL. The letters correspond to metabolites which were identified by co-elution with authentic standards. (a) (±)-*trans*-9,10-dihydrobenzo[*a*]pyrene-9,10-diol; (b) (±)-*trans*-4,5-dihydrobenzo[*a*]pyrene-4,5-diol; (c) (±)-*trans*-7,8-dihydrobenzo[*a*]pyrene-7,8-diol; (d) benzo[*a*]pyrene-6,12-dione; (e) 9-hydroxybenzo[*a*]pyrene; (f) 3-hydroxybenzo[*a*]pyrene. The metabolite fractions used for quantitation are bracketed. The ordinate shows dpm × $10^{-3}$ of [$^3$H].

To test for nonenzymatic oxidation of BAP, 25 ml of perfusate or 25 ml of lung homogenate with 2.5 nmole of [$^3$H]BAP was incubated for 45 min at 4°C. In both cases only the quinone fraction was increased, but this represented less than 5% of the quantity of quinones formed in control perfusion studies. Moreover, the addition of ascorbic acid (1.0 mM) to control samples prior to extraction did not affect the quantity of quinones formed.

*Determination of covalent binding of [$^3$H]benzo[a]pyrene in the isolated, ventilated, and perfused lung.* Total covalent binding was studied in trichloroacetic acid (TCA) precipitates of lung homogenates (Seikevitz, 1952). Briefly, 5 ml of lung homogenate was precipitated with an equal volume of 10% TCA, centrifuged, and decanted. The pellet was washed once with 4% TCA, once with 95% EtOH, twice with 2 vol ethanol:ether:chloroform (2:2:1), once with ether, and dried. The residue was dissolved in 5 ml of 2.5 N NaOH and 0.1 ml was taken for determination of radioactivity. Results were expressed as disintegrations per minute per milligram of protein (Lowry *et al.*, 1951).

*Pretreatment of rats with β-naphthoflavone.* To study the inducibility of BAP metabolism in the rat lung, rats were injected intraperitoneally with β-naphthoflavone (80 mg/kg) dissolved in 0.5 ml of stripped corn oil (Eastman Kodak Company, Rochester, N.Y.) 48 hr before removing the lungs for perfusion. Control animals were injected with 0.5 ml of stripped corn oil.

*Effects of metyrapone and indomethacin on [$^3$H]benzone[a]pyrene metabolism.* Metyrapone (50 μmole) was dissolved in 50 ml of perfusate and perfusion was

begun with this solution 1 min prior to infusion of [$^3$H]BAP. Indomethacin (5 μmole) was dissolved in 100 μl of DMSO and infused into the arterial circulation of the IVPL 10 min prior to the infusion of [$^3$H]BAP. The remainder of the experiment and analysis was as described above.

*Intratracheal administration of heavy metal salts.* Following cannulation of the trachea, 1 μmole of $NiCl_2$, $CdCl_2$, or $CoCl_2$ dissolved in 100 μl of isotonic sucrose (300 mM, pH 7.40) was instilled intratracheally *in vivo* at the point of maximum expiration. Absorption and distribution of the heavy metal salt solutions were allowed to continue for 5 min *in vivo*. The lungs were then removed and the IVPL was prepared as before. Control rats were treated in the same manner with isotonic sucrose alone. This dose of metal salts has been determined to alter the metabolism of *p*-nitroanisole in perfused rat lungs without altering lung function or causing edema (Williams and Menzel, 1982).

*Statistical methods.* Significant differences between metabolite groups obtained from control and experimental perfused lungs were determined by the two-sided *t* test.

## RESULTS

*Induction of [$^3$H]benzo[a]pyrene metabolism by pre-treatment with β-naphthoflavone.* The effect of BNF pretreatment of the metabolism of [$^3$H]BAP is shown in Table 1. Following pretreatment with BNF, the quantity of unmetabolized [$^3$H]BAP is 16% of corn oil-treated controls. A larger fraction of the metabolites was highly polar and not extractable with organic solvent. More polar metabolites are formed as judged by an increase in the total recoverable metabolites (free plus glucuronide conjugates) in the polar fraction and a decrease in the total phenol fraction, compared to controls. Glucuronide conjugates are detectable in the polar and diol 1 fractions following BNF pretreatment but are not evident in control lungs. Total glucuronide conjugates formed also increases following BNF treatment. Total glucoronide conjugates represent 35.7% of the total extractable metabolites from BNF-induced lungs, compared to 14.7% in control lung. No sulfate conjugates were detectable in the perfusates or tissue homogenates of lungs from control or BNF pretreated rats. BNF pretreatment also results in a 60% increase over controls in the quantity of covalently bound radioactivity (see Table 4).

*Inhibition of [$^3$H]benzo[a]pyrene metabolism by metyrapone.* The metabolism of [$^3$H]BAP is inhibited by 1.0 mM metyrapone (Table 2). The quantity of metabolites extractable from the perfusate and tissue is less in all metabolite fractions except the quinone. Most of the radioactivity is in the form of unmetabolized BAP. The total extractable metabolites in metyrapone-treated lungs is 42% of control values while the quantity of unmetabolized [$^3$H]BAP is 345% of controls. Determination of conjugates was not performed in metyrapone treated lungs. The binding of [$^3$H]BAP metabolites to lung macromolecules in metyrapone treated lungs is 21.7% of control values (see Table 4).

*[$^3$H]Benzo[a]pyrene metabolism in the IVPL in the presence of indomethacin.* The effect of 0.1 mM indomethacin on the metabolite profile of [$^3$H]BAP in the IVPL is depicted in Table 3. Indomethacin causes a decrease in the formation of

TABLE 1

DISTRIBUTION OF [$^3$H]BENZO[*a*]PYRENE METABOLITES IN THE PERFUSATE AND TISSUE OF ISOLATED, VENTILATED, AND PERFUSED LUNGS FROM RATS PRETREATED WITH β-NAPHTHOFLAVONE

| Metabolite fraction | pmole [$^3$H]BAP equivalents/g wet lung[a] | | | | | |
|---|---|---|---|---|---|---|
| | Perfusate | | Lung | | Total | |
| | Corn oil | B-NF | Corn oil | B-NF | Corn oil | B-NF |
| Polar | 290.5 ± 45.7 | 276.7 ± 81.8 | 22.0 ± 9.0 | 83.5 ± 66.1 | 312.5 ± 44.2 | 360.2 ± 53.9 |
| As glucuronide | ND | 104.4 ± 56.9 | ND | ND | ND | 104.4 ± 56.9 |
| Diol 1 | 339.8 ± 21.7 | 122.6 ± 24.2[b] | 18.7 ± 4.0 | 91.2 ± 75.9 | 358.4 ± 17.8 | 247.1 ± 103.0 |
| As glucuronide | ND | 42.6 ± 25.2 | ND | ND | ND | 42.6 ± 25.2 |
| Diol 2 | 282.2 ± 30.5 | 144.6 ± 14.5[c] | 19.5 ± 6.7 | 62.2 ± 48.9 | 280.0 ± 12.0 | 206.8 ± 46.9 |
| As glucuronide | 32.4 ± 7.4 | 87.2 ± 22.1[d] | 7.2 ± 1.7 | 1.1 ± 0.2[e] | 39.6 ± 8.3 | 88.3 ± 22.0[d] |
| Quinone | 205.9 ± 33.4 | 51.7 ± 9.8[b] | 107.2 ± 30.1 | 34.0 ± 10.4 | 277.7 ± 49.2 | 85.7 ± 15.2[c] |
| As glucuronide | 84.0 ± 5.5 | 194.2 ± 65.9 | 21.7 ± 5.2 | 4.3 ± 2.2[d] | 105.8 ± 0.25 | 198.5 ± 63.8 |
| Phenol | 80.4 ± 8.1 | 24.3 ± 8.1 | 28.9 ± 13.1 | 19.6 ± 9.9 | 109.4 ± 18.6 | 43.9 ± 5.7[c] |
| As glucuronide | 70.2 ± 12.7 | 84.0 ± 5.7 | 18.3 ± 6.6 | 4.6 ± 2.7 | 88.4 ± 16.8 | 88.6 ± 8.3 |
| Total organic soluble metabolites | 1,198.8 ± 103.3 | 653.2 ± 49.7[c] | 161.0 ± 35.0 | 290.5 ± 202.7 | 1,359.8 ± 69.9 | 937.5 ± 148.5 |
| Total glucuronides | 186.6 ± 25.4 | 512.4 ± 144.5[d] | 47.2 ± 8.7 | 11.3 ± 4.4[c] | 233.8 ± 24.9 | 523.6 ± 141.1[d] |
| Total metabolites | 1,385.5 ± 116.5 | 1,165.6 ± 105.8 | 208.2 ± 43.0 | 301.8 ± 200.2 | 1,593.6 ± 73.4 | 1,467.3 ± 295.9 |
| Unmetabolized [$^3$H]BAP | 625.9 ± 35.9 | 120.3 ± 10.5[b] | 415.2 ± 30.1 | 51.9 ± 14.0[b] | 1,041.1 ± 61.3 | 172.2 ± 21.9[b] |

[a] Each value represents the mean ± SE of 3 determinations.
[b] $P \leq 0.001$.
[c] $P \leq 0.01$.
[d] $P \leq 0.05$.
[e] $P \leq 0.02$.

TABLE 2

DISTRIBUTION OF [$^3$H]BENZO[*a*]PYRENE METABOLITES IN THE PERFUSATE AND TISSUE OF ISOLATED, VENTILATED RAT LUNGS PERFUSED WITH 1.0 mM METYRAPONE

| Metabolite fraction | pmole [$^3$H]BAP equivalents/g wet lung | | | | | |
|---|---|---|---|---|---|---|
| | Perfusate | | Lung | | Total | |
| | Control[a] | Metyrapone[b] | Control[a] | Metyrapone[b] | Control[a] | Metyrapone[b] |
| Polar | 171.8 ± 57.3 | 49.5 ± 23.4[c] | 20.8 ± 6.9 | 7.9 ± 1.3[c] | 192.6 ± 62.8 | 57.4 ± 24.8[c] |
| Diol-1 | 254.6 ± 27.3 | 23.0 ± 4.2[d] | 35.2 ± 9.5 | 10.2 ± 5.4[c] | 289.8 ± 20.3 | 33.2 ± 7.7[d] |
| Diol-2 | 221.7 ± 30.3 | 76.2 ± 21.9[e] | 39.5 ± 17.6 | 13.1 ± 7.5[c] | 261.9 ± 26.1 | 89.3 ± 15.6[e] |
| Quinone | 148.5 ± 27.0 | 146.0 ± 44.0 | 46.1 ± 13.7 | 58.4 ± 10.5 | 194.8 ± 16.4 | 204.4 ± 53.1 |
| Phenol | 80.0 ± 15.1 | 47.7 ± 14.9[c] | 64.2 ± 19.8 | 20.9 ± 2.9[e] | 144.3 ± 26.3 | 68.6 ± 16.9[c] |
| Total organic soluble metabolites | 876.6 ± 64.8 | 342.4 ± 106.0[e] | 205.8 ± 55.5 | 110.5 ± 5.3[d] | 1,082.5 ± 15.6 | 452.9 ± 109.8[d] |
| Unmetabolized [$^3$H]BAP | 722.9 ± 32.1 | 2021.2 ± 147.9 | 543.1 ± 41.2 | 2,345.0 ± 524.8[d] | 1,266.0 ± 80.2 | 4,366.2 ± 394.1[d] |

[a] Each value represents the mean ± SE of 4 determinations.
[b] Each value represents the mean ± SE of 3 determinations.
[c] $P \leq 0.05$.
[d] $P \leq 0.001$.
[e] $P \leq 0.01$.

TABLE 3

DISTRIBUTION OF [$^3$H]BENZO[*a*]PYRENE METABOLITES IN THE PERFUSATE AND TISSUE OF ISOLATED, VENTILATED RAT LUNGS PERFUSED WITH 0.1 mM INDOMETHACIN

| Metabolite fraction | pmole [$^3$H]BAP equivalents/g wet lung[a] | | | | | |
|---|---|---|---|---|---|---|
| | Perfusate | | Lung | | Total | |
| | Control | Indomethacin | Control | Indomethacin | Control | Indomethacin |
| Polar | 171.8 ± 57.3 | 96.1 ± 6.8 | 20.8 ± 6.9 | 23.7 ± 1.4 | 192.6 ± 62.8 | 119.8 ± 6.4 |
| As glucuronide | ND | ND | ND | ND | ND | ND |
| Diol 1 | 254.6 ± 27.3 | 184.2 ± 8.2[b] | 35.2 ± 9.5 | 43.2 ± 4.8 | 289.8 ± 20.3 | 227.4 ± 11.4[b] |
| As glucuronide | ND | ND | ND | ND | ND | ND |
| Diol 2 | 221.7 ± 30.3 | 184.4 ± 11.1 | 39.5 ± 17.6 | 73.4 ± 20.0 | 261.2 ± 26.1 | 257.8 ± 29.0 |
| As glucuronide | 23.0 ± 12.6 | 20.8 ± 9.9 | 8.2 ± 3.2 | 3.8 ± 0.6 | 31.1 ± 10.5 | 24.6 ± 10.3 |
| Quinone | 148.5 ± 27.0 | 194.9 ± 14.0 | 46.1 ± 13.7 | 117.4 ± 12.4[c] | 194.6 ± 16.4 | 313.2 ± 17.9[c] |
| As glucuronide | 47.2 ± 18.9 | 72.7 ± 26.1 | 16.2 ± 1.8 | 19.7 ± 2.6 | 63.4 ± 20.2 | 92.4 ± 28.6 |
| Phenol | 80.0 ± 15.1 | 59.8 ± 4.1 | 64.2 ± 19.8 | 41.9 ± 5.7 | 144.3 ± 26.3 | 101.7 ± 3.4 |
| As glucuronide | 56.4 ± 21.3 | 17.4 ± 10.3 | 30.9 ± 6.8 | 8.3 ± 2.7[c] | 87.3 ± 23.9 | 25.7 ± 12.7[b] |
| Total organic soluble metabolites | 876.6 ± 64.8 | 719.4 ± 13.9[b] | 205.8 ± 55.5 | 299.5 ± 40.9 | 1,082.4 ± 15.6 | 1,018.9 ± 54.2 |
| Total glucuronides | 126.6 ± 51.1 | 110.8 ± 43.9 | 55.3 ± 9.4 | 31.7 ± 5.5 | 181.9 ± 54.1 | 137.5 ± 52.5 |
| Total metabolites | 987.3 ± 77.2 | 830.2 ± 37.7 | 261.1 ± 59.7 | 331.2 ± 37.7 | 1,248.4 ± 61.8 | 1,161.4 ± 39.9 |
| Unmetabolized [$^3$H]BAP | 722.9 ± 32.1 | 1,545.6 ± 57.9[d] | 543.1 ± 41.2 | 1,480.3 ± 105.2 | 1,266.0 ± 80.2 | 3,025.9 ± 141.4[d] |

[a] Each value represents the mean ± SE of 4 determinations.
[b] $P \leq 0.05$.
[c] $P \leq 0.01$.
[d] $P \leq 0.001$.

TABLE 4

EFFECTS OF VARIOUS CHEMICAL MODULATORS OF DRUG METABOLISM ON THE BINDING OF [$^3$H]BAP METABOLITES TO TISSUE MACROMOLECULES IN THE ISOLATED, VENTILATED, AND PERFUSED RAT LUNG

| Treatment | Number of experiments | Binding (dpm/μg protein) | % of Control |
|---|---|---|---|
| Control | 4 | 1060.0 ± 72.2 | — |
| Metyrapone (1.0 mM) | 3 | 230.4 ± 132.5[a] | 21.7 |
| Indomethacin (0.1 mM) | 4 | 847.0 ± 94.1[b] | 79.9 |
| Corn oil (0.5 ml ip) | 3 | 1062.8 ± 105.8 | NS |
| BNF (80 mg/kg ip) | 3 | 1695.0 ± 211.9[c] | 159.9 |

[a] $P \leq 0.001$.
[b] $P \leq 0.05$.
[c] $P \leq 0.02$.

metabolites in the diol-1 and phenol fractions. Total phenols (organic soluble plus glucoronide conjugates) are significantly lower in indomethacin-treated lungs compared to controls ($p \leq 0.01$). These decreases are accompanied by an increase in the total recoverable quinones formed. In the presence of indomethacin, 239% more [$^3$H]BAP remains unmetabolized compared to controls. Covalent binding of [$^3$H]BAP metabolites to lung tissue in the presence of indomethacin is 79.9% of controls (Table 4).

*Effects of intratracheal instillations of $NiCl_2$, $CdCl_2$, and $CoCl_2$ on [$^3$H]benzo[a]pyrene metabolism in the IVPL.* Intratracheal installations of 1 μmole per lung of $NiCl_2$ or $CdCl_2$ alter the metabolism of [$^3$H]BAP in the IVPL as reflected in the metabolite profiles (Table 5), the extent of metabolism of [$^3$H]BAP (Table 5), and in the amount of covalent binding to lung macromolecules (Table 6). The quantity of total extractable metabolites formed in the presence of $NiCl_2$ is significantly less than control values. The inhibition of metabolism is reflected largely in the total quantity of metabolites recoverable from the perfusate. Few differences are apparent in the amount of metabolites recoverable from lung homogenates. (Individual data for perfusate and tissue metabolites are not shown). While the quantity of metabolites in each fraction is less following $NiCl_2$ treatment, the mean values for each metabolite fraction are significantly different only when expressed as the total of all metabolites formed (Table 5). The overall inhibition by $NiCl_2$ of the metabolism of [$^3$H]BAP in the IVPL is more obvious as an increase in the amount of BAP which remains unmetabolized (Table 5). The covalent binding of [$^3$H]BAP metabolites is slightly, but not significantly, decreased by $NiCl_2$ (Table 6). Total glucuronide conjugates formed, expressed as percentages of the total recoverable metabolites, are 14.7 and 11.6% for control and $NiCl_2$ treated lungs, respectively.

$CdCl_2$, in comparison to $NiCl_2$, stimulates the metabolism of [$^3$H]BAP. The quantity of total extractable metabolites found in the perfusates and homogenates from $CdCl_2$-dosed lungs is greater than that found in control lungs (Table 5). This is particularly evident from the quantity of metabolites found in the perfusate (not shown). The total of all metabolites formed is twice as great in $CdCl_2$-treated lungs as in control lungs. $CdCl_2$ exerts no measurable effect upon glucuronide formation, however, since 13.6% of the total recoverable metabolites are conju-

TABLE 5

DISTRIBUTION OF TOTAL [$^3$H]BENZO[*a*]PYRENE METABOLITES IN THE PERFUSATE AND TISSUE OF ISOLATED, VENTILATED, AND PERFUSED RAT LUNGS TREATED WITH 1 μmole OF $NiCl_2$, $CdCl_2$, OR $CoCl_2$

| Metabolite fraction | Total pmole [$^3$H]BAP equivalents/g wet lung | | | |
|---|---|---|---|---|
| | Control[a] | $NiCl_2$[b] | $CdCl_2$[c] | $CoCl_2$[b] |
| Polar | 192.6 + 62.8 | 125.9 + 10.3 | 582.6 + 145.5[d] | 264.8 + 25.3 |
| Diol 1 | 289.8 + 20.3 | 238.5 + 28.4 | 585.1 + 106.2[d] | 252.8 + 57.7 |
| Diol 2 | 261.2 + 26.1 | 190.9 + 24.1 | 441.5 + 77.7[d] | 220.2 + 38.7 |
| As glucuronide | 31.2 + 10.5 | 20.2 + 4.4 | 50.1 + 24.4 | 40.9 + 16.6 |
| Quinone | 194.6 + 16.4 | 164.1 + 8.4 | 351.7 + 63.5[d] | 212.6 + 34.3 |
| As glucuronide | 63.4 + 20.2 | 44.8 + 18.0 | 177.2 + 39.9[d] | 74.5 + 18.8 |
| Phenol | 144.2 + 26.3 | 139.9 + 26.8 | 328.0 + 70.0[d] | 128.2 + 35.0 |
| As glucuronide | 87.3 + 23.9 | 47.3 + 8.2 | 134.2 + 26.1 | 80.2 + 20.5 |
| Total organic soluble metabolites | 1082.4 + 15.6 | 859.4 + 78.3[e] | 2287.8 + 455.0[d] | 1078.5 + 82.1 |
| Total glucuronide conjugates | 181.9 + 54.1 | 112.3 + 21.0 | 361.4 + 88.5 | 195.7 + 52.6 |
| Total metabolites | 1248.4 + 61.8 | 971.7 + 71.7[e] | 2649.1 + 512.4[d] | 1274.1 + 64.8 |
| Unmetabolized [$^3$H]BAP | 1266.0 + 80.2 | 1825.0 + 120.0[f] | 856.1 + 68.1[f] | 1062.2 + 130.2 |

[a] Mean ± S.E. of 4 lungs.
[b] Mean ± S.E. of 3 lungs.
[c] Mean ± S.E. of 5 lungs.
[d] $P \leq 0.05$.
[e] $P \leq 0.02$.
[f] $P \leq 0.01$.

gated. The stimulatory effect of $CdCl_2$ is also reflected in the quantity of unmetabolized [$^3$H]BAP. In the presence of $CdCl_2$, the quantity of [$^3$H]BAP remaining is 32% less than in control determinations. The increase in metabolism effected by $CdCl_2$ is correlated with increased covalent binding of [$^3$H]BAP metabolites to TCA precipitates of lung tissue (Table 6).

Dosing lungs with 1 μmole of $CoCl_2$ has no measurable effect on the metabolism or binding of [$^3$H]BAP.

## DISCUSSION

Previous studies have shown that the intact mammalian lung is capable of metabolizing BAP to water soluble metabolites and to products which bind co-

TABLE 6

EFFECTS OF INTRATRACHEAL INSTILLATION OF 1 μmole OF $NiCl_2$, $CdCl_2$, OR $CoCl_2$ ON THE BINDING OF [$^3$H]BAP METABOLITES IN THE ISOLATED, VENTILATED, AND PERFUSED RAT LUNG

| Treatment | Number of experiments | Binding (dpm/μg protein) | % of Control |
|---|---|---|---|
| Control | 4 | 1,060.0 ± 72.2 | — |
| $NiCl_2$ | 3 | 991.0 ± 41.0 | NS |
| $CdCl_2$ | 5 | 1,640.1 ± 217.4[a] | 154.7 |
| $CoCl_2$ | 3 | 1,013.4 ± 256.3 | NS |

[a] $P \leq 0.05$.

valently to lung macromolecules (Cohen *et al.*, 1977; Vainio *et al.*, 1976). The results presented here demonstrate that the rat lung metabolizes [$^3$H]BAP by an inducible enzyme system, active at basal levels, which is inhibited largely by metyrapone, a known inhibitor of cytochrome *P*-450. Furthermore, the basal activity is acutely sensitive to Ni and Cd, components of some atmospheric particulates with which PAH are also associated.

Qualitatively, the organic solvent extractable metabolites formed in the IVPL are similar to those formed in other test systems, as identified by co-chromatography with authentic standards. Control lung preparations are able to metabolize approximately 80% of the 5-nmole dose of BAP in 45 min to a variety of phenols, quinones, dihydrodiols, and more polar metabolites.

As in rat liver microsomes, the major metabolites formed upon incubation of BAP with rat lung microsomes are the BAP phenols (Uotila *et al.*, 1977), which represent 50 to 60% of total extractable metabolites. Similar results have been obtained in an isolated, perfused rat lung preparation when BAP was given by intratracheal instillation (Cohen *et al.*, 1977). The present results show that only about 20% of the total radioactivity is associated with the phenol fraction, and 40% of the metabolites in this fraction are glucuronide conjugates. In a relatively long-term perfusion under nonsaturating conditions, as is the case in our studies, the BAP phenols and diols are likely to be metabolized further by the pulmonary MFO system to more polar or conjugated products (Holder, 1977). The appearance of the large polar fraction observed in these studies (representing 15% of the total recoverable metabolites) is probably the result of extensive metabolism of primary [$^3$H]BAP metabolites by the pulmonary MFO system. It is also possible that epoxide hydratase hydrolysis, resulting in the formation of dihydrodiols, is a major determinant of arene oxide metabolism in the intact organ and that this pathway is favored over molecular rearrangement to phenols under the present conditions.

Under conditions of enzyme induction following BNF pretreatment, more polar diols and fewer phenols are formed compared to vehicle-treated controls. Epoxide hydratase is likely to be moderately induced under these conditions (Oesch *et al.*, 1973), as is UDP-glucuronyltransferase (Dutton, 1975). The increased formation of highly water soluble metabolites in BNF-induced lungs may reflect the ability of the lung to form glutathione conjugates with BAP arene oxide intermediates. Glutathione conjugation has been shown to be a significant pathway of BAP metabolism in the isolated, perfused rabbit lung (Ball *et al.*, 1979). However, the extent of glutathione conjugation was not measured in these studies. Sulfation was found not to be a major pathway of conjugation in this system, since no sulfatase sensitive metabolites were detected. Only a moderate increase in total covalent binding occurs following induction.

It is not clear from our studies whether indomethacin inhibition of [$^3$H]BAP metabolism results from inhibition of pulmonary cyclooxygenase or competitive inhibition for cytochrome *P*-450-dependent metabolism. Cyclooxygenase catalyzes the cooxygenation of BAP and archidonic acid (Sivarajah *et al.*, 1978). In rats and man, a major metabolite of indomethacin is desmethylindomethacin (Duggan and Duncan, 1970; Duggan *et al.*, 1972), suggesting that indomethacin

may be a substrate for *O*-demethylating enzymes in the lung. Itakura *et al.* (1979) have demonstrated an active *O*-demethylase system to be present in the rabbit and rat lung and have shown this to be an MFO. In guinea pig lung microsomes, however, indomethacin does not inhibit NADPH-dependent metabolism of BAP at concentrations up to 200 μM (Sivarajah *et al.*, 1978). In this same preparation, the major metabolic products formed in the presence of arachidonic acid and in the absence of NADPH are BAP-quinones. Formation of quinones in guinea pig lung microsomes is inhibited by indomethacin. In the present studies, quinone formation is increased in the presence of indomethacin. The reasons for this effect are not clear, but there are important differences between microsomal preparations and whole organs in the control of BAP metabolism. In our studies, the metabolism of [$^3$H]BAP occurs in the presence of endogenous levels of arachidonic acid and NADPH while the microsomal preparations contain saturating concentrations of these co-factors. In whole cells and organs, NADPH availability may control MFO activity (Thurman and Kauffman, 1980). More than one cytochrome *P*-450 may exist, so two simultaneous oxidations could deplete the available NADPH. Cytoplasmic enzyme systems (e.g., lipoxygenase and glutathione peroxidase) are not present in isolated microsomes. The influence of such factors on the metabolism of [$^3$H]BAP is not known. Our experiments suggest that quinones are not formed in the whole lung by the prostaglandin synthesizing system. Since quinone formation is neither induced by BNF pretreatment nor inhibited by metyrapone, quinones are formed by an enzyme system other than MFO in the lung or, more possibly, are formed nonenzymatically through the autoxidation of transient free radical intermediates.

While a mechanism for the effects of $NiCl_2$ and $CdCl_2$ on the pulmonary metabolism of [$^3$H]BAP cannot be derived from these studies, these metals alter this activity. Similar effects of these metals at identical doses on the pulmonary metabolism of *p*-nitroanisole in the perfused rat lung have been reported (Williams and Menzel, 1982). The inhibitory effect of $Fe_2O_3$ or crude air particulate matter on BAP metabolism in perfused rabbit lungs has been demonstrated (Warshawsky *et al.*, 1978).

The stimulation of metabolism of $CdCl_2$ is of obvious toxicological importance, since binding of reactive metabolites to lung macromolecules increases also. The alterations in BAP metabolism reported here are specific for $NiCl_2$ and $CdCl_2$, since $CoCl_2$ has no effect. Only a small fraction of the BAP was bound to macromolecules despite a large increase in overall metabolism. Most likely, $CdCl_2$ treatment results in inhibition of metabolite conjugation with glutathione. Formation of glucuronides was not affected by $CdCl_2$ despite increased BAP metabolism. The glutathione conjugation of styrene oxide in rat liver microsomes is inhibited by $10^{-4}$M $Cd^{2+}$ (Aitio *et al.*, 1978). However, in these same studies, this concentration of $Cd^{2+}$ also inhibited the hydroxylation of BAP, ethoxycoumarin de-ethylation, and epoxide hydratase. None of these activities was altered by $Ni^{2+}$, $Co^{2+}$, or $Zn^{2+}$. Certainly, in the present studies, $CdCl_2$ exerts no inhibitory effect upon the metabolism of [$^3$H]BAP. The profile of [$^3$H]BAP from $CdCl_2$-dosed lungs suggests that inhibition of epoxide hydrase activity is not a factor, since polyhydroxylated metabolites increase to the same extent as phenols. If BAP is

also metabolized by cyclooxygenase, $CdCl_2$ treatment does not inhibit this activity either.

Inhibition of [$^3$H]BAP metabolism by $NiCl_2$ treatment does not appreciably affect binding of BAP metabolites to lung macromolecules. Metabolites are readily released by the lung, so Ni exposure could result in the translocation of more unmetabolized parent compound, as well as metabolites from the lung to other target organs for further metabolism. Inhibition of pulmonary BAP metabolism could result in an effective increase in the residence time of inhaled organic pollutants in the lung and allow redistribution of the substrate within pulmonary tissue. Slower metabolism does not decrease covalent binding so the hazard for exposure of the organ is not decreased. This, then, ultimately may increase the probability of a local toxic effect.

Pulmonary metabolism of inhaled PAH may be critical in the initiation of lung tumors. The coexistence of heavy metals with PAH in environmental aerosols suggests that the carcinogenic potency of such aerosols may depend upon both the PAH and the heavy metal content. The observed carcinogenesis of heavy metals may, in fact, be mediated partially through their interplay with organic carcinogens.

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## REFERENCES

Aitio, A., Ahotupa, M., and Parkki, M. G. (1978). Inhibition of drug metabolizing enzymes by heavy metals *in vitro*. *Biochem. Biophys. Res. Commun.* **83**, 850–856.

Ball, L. M., Plummer, J. L., Smith, B. R., and Bend, J. R. (1979). Benzo(a)pyrene oxidation, conjugation and disposition in the isolated, perfused rabbit lung: Role of the glutathione S-transferases. *Med. Biol.* **57**, 298–305.

Baum, E. J. (1978). Occurrence and surveillance of polycyclic aromatic hydrocarbons. *In* "Polycyclic Hydrocarbons and Cancer" (H. V. Gelboin and P. O. P. Ts'o, Eds.), Vol. 1, pp. 45–70. Academic Press, New York.

Cohen, G. M., Uotila, P., Hartiala, J., Soulinna, E. M., Simberg, N., and Pelkonen, O. (1977). Metabolism and covalent binding of $^3$H-benzo(a)pyrene by isolated, perfused lung and short term tracheal organ culture of cigarette exposed rats. *Cancer Res* **37**, 47–55.

Duggan, D. E., and Duncan, A. E. W. (1970). The metabolism of indomethacin-$^{14}$C in normal and salicylate-treated rats. *Pharmacologist* **12**, 202.

Duggan, D. E., Hogans, A. F., Kwan, K. C., and McMahon, E. G. (1972). The metabolism of indomethacin in man. *J. Pharmacol. Exp. Ther.* **181**, 563–575.

Dutton, G. J. (1975). Control of UDP-glucuronyltransferase activity. *Biochem. Pharmacol.* **24**, 1835–1841.

Gelboin, H. V. (1969). A microsome-dependent binding of benzo(a)pyrene to DNA. *Cancer Res* **29**, 1272–1276.

Hayes, T. L., Pawley, J. B., and Fisher, G. L. (1978). The effect of chemical variability of individual fly ash particles on cell exposure. *Scanning Electron Microsc.* **1**, 239–244.

Heidelberger, C. (1975). Chemical carcinogenesis. *Annu. Rev. Biochem.* **44**, 79–121.

Holder, G. M., Yagi, H., Jerina, D. M., Levin, W., Lu, Y. H., and Conney, A. H. (1975). Metabolism of benzo(a)pyrene: Effect of substrate concentration and 3-methylcholanthrene pretreatment on hepatic metabolism by microsomes from rats and mice. *Arch. Biochem. Biophys.* **170**, 557–566.

Itakura, N., Fisher, A. B., and Thurman, R. G. (1979). Cytochrome P-450 linked p-nitroanisole O-demethylation in the perfused lung. *J. Appl. Physiol.* **43**, 238–245.

Kapitulnik, J., Levin, W., Conney, A. H., Yagi, H., and Jerina, D. M. (1977). Benzo(a)pyrene 7,8-dihydrodiol is more carcinogenic than benzo(a)pyrene in newborn mice. *Nature (London)* **266**, 378–380.

Kinoshita, N., and Gelboin, H. V. (1972). Aryl hydrocarbon hydroxylation in 7,12-dimethylbenzo(a)anthracene skin tumorigenesis: On the mechanism of 7,8-benzoflavone inhibition of tumorigenesis. *Proc. Natl. Acad. Sci. USA* **69**, 824–828.

Lee, R. E., Goranson, S. G., Enrione, R. E., and Morgan, G. B. (1972). National air surveillance cascade impactor network. II. Site distribution measurements of trace metal components. *Environ. Sci. Technol.* **6**, 1025–1030.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Maher, V. M., and McCormick, J. J. (1979). Mammalian cell mutagenesis by polycyclic aromatic hydrocarbons and their derivatives. *In* "Polycyclic Hydrocarbons and Cancer" (H. V. Gelboin and P. O. P. Ts'o, Eds.), Vol. 2, pp. 137–160. Academic Press, New York.

Menden, E. E., Elia, V. J., Michael, L. W., and Petering, H. G. (1972). Distribution of cadmium and nickel in tobacco during cigarette smoking. *Environ. Sci. Technol.* **6**, 830–832.

Nagao, M., and Sugimura, T. (1979). Mutagenesis: Microbial systems. *In* "Polycyclic Hydrocarbons and Cancer" (H. V. Gelboin and P. O. P. Ts'o, Eds.), Vol. 2, pp. 99–121. Academic Press, New York.

Natusch, D. F. S., and Wallace, J. R. (1974). Toxic trace elements: Preferential concentration in respirable particles. *Science* **183**, 202–204.

Nebert, D. W., Considine, N., and Owens, I. S. (1973). Genetic expression of aryl hydrocarbon hydroxylase induction. VI. Control of other aromatic hydrocarbon-inducible mono-oxygenase activities at or near the same genetic locus. *Arch. Biochem. Biophys.* **157**, 148–159.

Oesch, F., Morris, N., Daly, J. W., Gielen, J. E., and Nebert, D. W. (1973). Genetic expression of the induction of epoxide hydrase and aryl hydrocarbon hydroxylase activities in the mouse by phenobarbital or 3-methylchloranthrene. *Mol. Pharmacol.* **9**, 692–696.

Pierce, R. C., and Katz, M. (1975). Dependency of polynuclear aromatic hydrocarbon content on size distribution of atmospheric aerosols. *Environ. Sci. Technol.* **9**, 347–353.

Seikevitz, P. (1952). Uptake of radioactive alanine *in vitro* into the proteins of rat liver fractions. *J. Biol. Chem.* **195**, 549–565.

Sivarajah, K., Anderson, M. W., and Eling, T. E. (1978). Metabolism of benzo(a)pyrene to reactive intermediates via prostaglandin biosynthesis. *Life Sci.* **23**, 2571–2578.

Thurman, R. G., and Kauffman, F. C. (1980). Factors regulating drug metabolism in intact hepatocytes. *Pharmacol. Rev.* **31**, 229–251.

Uotila, P., Pelkonen, O., and Cohen, G. M. (1977). The effects of cigarette smoke on the metabolism of $^3$H-benzo(a)pyrene by rat lung microsomes. *Cancer Res.* **37**, 2156–2161.

Vainio, H., Uotila, P., Hartiala, J., and Pelkonen, O. (1976). The fate of intratracheally instilled benzo(a)pyrene in the isolated, perfused rat lung of both control and 20-methylcholanthrene pretreated rats. *Res. Commun. Chem. Pathol. Pharmacol.* **13**, 259–271.

Warshawsky, D., Niemeier, R. W., and Bingham, E. (1978). Influence of particulates on metabolism of benzo(a)pyrene in the isolated perfused lung. *In* "Carcinogenesis—A Comprehensive Survey" (P. W. Jones and R. I. Freudenthal, Eds.), Vol. 3, pp. 347–360. Raven Press, New York.

Williams, S. J., Holden, K. M., Sabransky, M., and Menzel, D. B. (1980). The distributional kinetics of $Ni^{2+}$ in the rat lung. *Toxicol. Appl. Pharmacol.* **55**, 85–93.

Williams, S. J., and Menzel, D. B. (1982). Modulation of pulmonary p-nitroanisole O-demethylase by intratracheally instilled heavy metal salts. *Toxicol. Appl. Pharmacol.* **64**, 431–438.